

Assessment of dietary vitamin D requirements during pregnancy and lactation^{1,2}

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ABSTRACT

Concerns about vitamin D have resurfaced in medical and scientific literature because the prevalence of vitamin D deficiency in the United States, particularly among darkly pigmented persons, has increased. The primary goals of this review were to discuss past and current literature and to reassess the dietary reference intake for vitamin D in adults, with particular focus on women during pregnancy and lactation. The appropriate dose of vitamin D during pregnancy and lactation is unknown, although it appears to be greater than the current dietary reference intake of 200–400 IU/d (5–10 $\mu\text{g}/\text{d}$). Doses of $\leq 10\,000$ IU vitamin D/d (250 $\mu\text{g}/\text{d}$) for up to 5 mo do not elevate circulating 25-hydroxyvitamin D to concentrations > 90 ng/mL, whereas doses < 1000 IU/d appear, in many cases, to be inadequate for maintaining normal circulating 25-hydroxyvitamin D concentrations of between 15 and 80 ng/mL. Vitamin D plays no etiologic role in cardiac valvular disease, such as that observed in Williams syndrome, and, as such, animal models involving vitamin D intoxication that show an effect on cardiac disease are flawed and offer no insight into normal human physiology. Higher doses of vitamin D are necessary for a large segment of Americans to achieve concentrations equivalent to those in persons who live and work in sun-rich environments. Further studies are necessary to determine optimal vitamin D intakes for pregnant and lactating women as a function of latitude and race. *Am J Clin Nutr* 2004;79:717–26.

KEY WORDS Vitamin D, cholecalciferol, calcifediol, pregnancy, breast milk, lactation

INTRODUCTION

The primary goal of this review was to discuss and begin to reassess the dietary reference intake (DRI) for vitamin D during pregnancy and lactation in women. This reassessment is critical because the current recommendations result in a high degree of vitamin D deficiency, especially in the African American population (1). This avenue of research already has begun in the healthy adult population (2, 3) and serves as a model for vitamin D supplementation during pregnancy and lactation. The history of dietary vitamin D requirements and recommendations, issues of toxicity and hypervitaminosis D, and the specific issues that pertain to pregnancy and lactation are highlighted in this review.

The first issue addressed is the definition of vitamin D. When we refer to vitamin D, we are speaking of the parent compound cholecalciferol—the form found in vitamin supplements and fortified dairy products and not the hormonal form of vitamin D, namely 1,25-dihydroxycholecalciferol. Thus, we do not discuss studies in which the focus is on the hormonal form of the vitamin,

because these studies are pharmacologic in nature and have no bearing on normal physiology. Rather, we focus on physiologically based studies that reevaluated the actual nutritional requirement for vitamin D during human pregnancy and lactation and that accounted for racial factors.

DIETARY REFERENCE INTAKE FOR VITAMIN D: WHAT IS THE EVIDENCE?

An excellent review by Vieth (4) addresses how arbitrary the determination of the vitamin D requirements in the general adult population was. In the next 3 paragraphs, we paraphrase from this review (4). Before 1997, the DRI for vitamin D in infants and children was 10 μg (400 IU) (5). In essence, the scientific basis for this dose was that it approximated what was in a teaspoon (≈ 5 mL) of cod-liver oil and had long been considered safe and effective in preventing rickets (6). The basis for adult vitamin D recommendations is even less well defined. Forty years ago, an expert committee on vitamin D provided only anecdotal support for what it referred to as “the hypothesis of a small requirement” for vitamin D in adults, and it recommended one-half the infant dose to ensure that adults obtain some from the diet (7). In England, an adult requirement of only 2.5 $\mu\text{g}/\text{d}$ (100 IU/d) was substantiated on the basis of findings in 7 adult women with severe nutritional osteomalacia whose bones showed a response when given this amount (8). The adult DRI of 5 $\mu\text{g}/\text{d}$ (200 IU/d) was described as a “generous allowance” in the 1989 version of American recommended dietary allowances (RDA; 5). What is truly remarkable is that the basis for these recommendations was made before it was possible to measure the circulating concentration of 25-hydroxyvitamin D [25(OH)D], the indicator of nutritional vitamin D status (9, 10).

LOWEST OBSERVED ADVERSE EFFECT LEVEL

Equally important with respect to daily vitamin D intakes is the lowest observed adverse effect level (LOAEL). Again, there is a lack of evidence to support statements about the toxicity of moderate doses of vitamin D. For instance, in the 1989 US RDA it is stated that 5 times the DRI for vitamin D may be harmful (5). This

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recommendation relates back to a 1963 expert committee report (6), which then refers back to the primary reference, a 1938 report in which linear bone growth was suppressed in infants given 45–158 μg (1800–6300 IU) vitamin D/d (11). The study was not conducted in adults and, thus, does not form a scientific basis for a safe upper limit in adults. The same applies to a statement in the 1987 council report from the American Medical Association: “dosages of 10 000 IU/d for several months have resulted in marked disturbances in calcium metabolism, and, in some cases—death.” Two references were cited to substantiate this claim. One reference was to a review article about vitamins in general, which gave no evidence for and cited no other reference for its claim of toxicity at vitamin D doses as low as 250 $\mu\text{g}/\text{d}$ (10 000 IU/d) (12). The other reference dealt with 10 patients with vitamin D toxicity reported in 1948, for whom the vitamin D dose was actually 3750–15 000 $\mu\text{g}/\text{d}$ (150 000–600 000 IU/d) (13); of note, all of the patients recovered. These same points were rehearsed in the 1990 Institute of Medicine Publication, *Nutrition During Pregnancy* (14). The issue of poorly substantiated claims of toxicity extends even to the most recent revision for vitamin D intakes published by the National Academy of Sciences (15).

The only study cited to address the question of critical end-point doses for vitamin D (potential adverse effect level) was one by Narang et al (16). The current no observed adverse effect level (NOAEL) of 50 $\mu\text{g}/\text{d}$ (2000 IU/d) is based on the finding of Narang et al of a mean serum calcium concentration $> 11 \text{ mg}/\text{dL}$ in the 6 “normal” subjects given 95 μg (3800 IU) vitamin D/d; this intake became the LOAEL. The next lowest test dose used by Narang et al, 60 $\mu\text{g}/\text{d}$ (2400 IU/d), with 20% less as the safety margin, became the NOAEL. Narang et al reported only serum electrolyte changes; the doses of vitamin D were not verified, and circulating 25(OH)D concentrations were not reported. It is unfortunate that the National Academy of Sciences based any recommendation on such a limited study. Recent reports by Vieth et al (2) and Heaney et al (3) have proven the above claims to be incorrect. As originally stated by Vieth (4), we have yet to find published evidence of toxicity in adults from an intake of 250 $\mu\text{g}/\text{d}$ (10 000 IU/d) that is verified by the circulating 25(OH)D concentration. The DRI, LOAEL, and NOAEL for vitamin D in adult humans have been established with insufficient scientific evidence and thus require correction through sound scientific studies.

DIETARY REFERENCE INTAKE FOR INFANTS, CHILDREN, AND ADULTS ON A PER-KILOGRAM BASIS

The question that has intrigued our group for years is the following: How is it possible that the DRI for vitamin D is the same for a 1-kg premature human infant, a 3.5-kg term infant, and a 90-kg adult? The recommendation for all of three of these groups is 400 IU/d (10 $\mu\text{g}/\text{d}$)! To answer this question, it is necessary to ascertain the effect of a daily intake of 400 IU vitamin D/d on circulating 25(OH)D concentrations in infants and adults. We published the results of a study in infants more than a decade ago (17). In that study, term (weight: $3.4 \pm 0.4 \text{ kg}$; $\bar{x} \pm \text{SD}$) and preterm ($1.3 \pm 0.2 \text{ kg}$) infants were supplemented with 400 IU (10 μg) vitamin D/d for 4 mo. The circulating 25(OH)D concentration (in ng/mL), the nutritional indicator of vitamin D status, increased during this period in the term (from

11 ± 9 to $26 \pm 12 \text{ ng}/\text{mL}$; $\bar{x} \pm \text{SD}$) and preterm (from 11 ± 5 to $51 \pm 19 \text{ ng}/\text{mL}$) infants. These are healthy increases; thus, a daily dose of 400 IU (10 μg) vitamin D appears to be effective in raising the vitamin D concentration to the accepted normal range for infants (15–80 ng/mL).

What effect does a daily dose of 400 IU vitamin D for an extended time (months) have in adults? The answer is little or nothing. At this dose (10 $\mu\text{g}/\text{d}$) in an adult, circulating 25(OH)D concentrations usually remain unchanged or decline. This was first shown in both adolescent girls and young women (18, 19). So the question is, what vitamin D intake is required to maintain or preferably improve the nutritional vitamin D status in adults in general and in pregnant or lactating adults specifically? This is a complex scientific question, yet recent well-controlled studies have provided some provisional answers (2, 3). First, what is the “normal” circulating concentration of 25(OH)D in the adult population? Data taken from the Mayo Medical Laboratories in Rochester, MN, list the normal range to be 15–80 ng/mL (20). This range is in accordance with what we have found in our laboratory; however, the circulating 25(OH)D concentration is dependent on season and latitude, as evidenced by the reference ranges (21). In sun-rich environments, circulating 25(OH)D ranges from 54 to 90 ng/mL (22–24).

NORMATIVE ADULT DATA IN A SUN-RICH ENVIRONMENT

Humans have evolved at exposures of $> 20\,000 \text{ IU}$ (500 μg) vitamin D/d from the sun. In fact, a 0.5-h exposure to the summer sun between 1000 and 1400 in a bathing suit (≈ 3 times the minimal erythemal dose) will initiate the release of $\approx 50\,000 \text{ IU}$ (1.25 mg) vitamin D into the circulation within 24 h of exposure in white persons (25). African Americans require up to 5 times this solar exposure to achieve the same response (26, 27). In whites who have a deep tan because of melanin deposition in the skin, the response is $\approx 50\%$ of that stated above, ie, only $\approx 20\,000$ – $30\,000 \text{ IU}$ (500–750 μg) vitamin D will be liberated (28). Finally, if wearing clothing or total body sunscreen, the cutaneous release of vitamin D is completely blunted (24, 29–31). So, in light of the above facts, a DRI of 400 IU/d (10 $\mu\text{g}/\text{d}$) in adults seems woefully inadequate to maintain normal circulating concentrations of vitamin D in adults with minimal solar exposure.

VITAMIN D INTAKES REQUIRED TO SUSTAIN AN ADEQUATE NUTRITIONAL STATUS OF VITAMIN D

On the basis of 25(OH)D concentrations in sun-replete adults, what vitamin D intake is required to sustain an adequate nutritional status of vitamin D? In whites who experience significant solar exposure to the body routinely, this is not an important question. As a population, however, our unprotected exposure to the sun is declining rapidly because of the fear of skin cancer and premature aging resulting from public education campaigns. For persons with darker pigmentation, the answer is more complicated. The darker pigmentation of the African American population is a powerful natural sunscreen, which adversely affects cutaneous vitamin D synthesis.

The first study to address this topic was published by Vieth et al in 2001 (2). In this study, the investigators supplemented healthy adults daily with either 25 μg (1000 IU) or 100 μg (4000

TABLE 1Summary of high-dose vitamin D supplementation studies in healthy adults and pregnant and lactating women¹

Reference ²	Subject type	No. of subjects	Vitamin D dose	Therapy duration	Initial 25(OH) D	Endpoint 25(OH)D	Actual change in 25(OH)D	Theoretical change in 25(OH)D ³
			IU/d	mo	ng/mL	ng/mL	ng/mL	ng/mL
Brooke et al, 1980 (32) ⁴	Pregnant	67 Control	0	3	8.0	6.5	-1.5	—
	Asians	59 Supplemented	1000		8.0	67.2	+59.2	+7.0
Cockburn et al, 1980 (33)	Pregnant women	82 Control	0	4	13.0	13.0	0	—
		82 Supplemented	400		15.6	17.1	+1.5	+2.8
Delvin et al, 1986 (34)	Pregnant women	13 Supplemented	1000	3	13 ± 4 ⁵	26 ± 7	+13	+7.0
Mallet et al, 1986 (35)	Pregnant women	29 Supplemented	1000	3	3.8 ± 2.0	10.1 ± 6.3	+6.3	+7.0
Ala-Houhala, 1985 (36)	Lactating women	16 Supplemented	1000	4.5	10	26	+16	+7.0
		17 Supplemented	2000		13	36	+23	+14.0
Vieth et al, 2001 (2)	Healthy males and females	10 M, 23 F	1000	5	16.3 ± 6.2	27.5 ± 6.8	+11.2	+7.0
		10 M, 23 F	4000		18.7 ± 7.1	38.6 ± 5.8	+19.9	+28.0
Datta et al, 2002 (37)	Pregnant minorities	80 Supplemented	800–1600	>6	5.8 ± 0.9	11.2 ± 6.3	+5.4	+5.6 → 11.2
Heaney et al, 2003 (3)	Healthy males	67 Supplemented	200	5	28.0 ± 9.4	23.0 ± 7.1	-5.0	+1.4
			1000		28.8 ± 6.4	33.6 ± 6.5	+4.8	+7.0
			5000		27.7 ± 6.7	64.5 ± 15	+36.8	+35.0
			10 000		26.2 ± 9.7	90.0 ± 25	+63.8	+70.0
Hollis and Wagner, in press (38)	Lactating women	9 Supplemented	2000	3	27.6 ± 9.8	36.1 ± 7.0	+8.5	+14.0
		9 Supplemented	4000		32.6 ± 6.9	44.5 ± 11.4	+11.9	+28.0

¹ 25(OH)D, 25-hydroxyvitamin D.² Sunlight exposure was not discussed in the studies by Brooke et al, Cockburn et al, Delvin et al, Mallet et al, and Datta et al. Minimal sunlight exposure was controlled for in the studies by Ala-Houhala, Vieth et al, Heaney et al, and Hollis and Wagner.³ The calculations were based on a linear regression model from Heaney et al (3).⁴ It is very likely that the wrong dose was reported. The response observed is one that would be expected after supplementation with 10000 IU/d for 3 mo.⁵ $\bar{x} \pm SD$ (all such values).

IU) vitamin D for 5 mo. Circulating 25(OH)D concentrations increased from 16.3 ± 6.2 to 27.5 ± 6.8 ng/mL and from 18.7 ± 6.0 to 38.6 ± 5.8 ng/mL in the 1000- and 4000-IU groups, respectively. Not a single adverse event or episode of hypercalciuria was observed in the 60 subjects enrolled in the study. In an even more detailed report, Heaney et al (3) studied 67 men divided into 4 groups that received 200 IU (5 μ g), 1000 IU (25 μ g), 5000 IU (125 μ g), or 10 000 IU (250 μ g) vitamin D/d for 5 mo. The 200-IU/d group failed to maintain circulating 25(OH)D concentrations during the study period. The remaining 3 groups responded in a dose-response fashion with respect to elevations in circulating 25(OH)D concentrations. From these data, with the use of regression analysis, it has become possible to calculate a response of circulating 25(OH)D from a given oral intake of vitamin D. The data show that for every 1 μ g (40 IU) of vitamin D intake, circulating 25(OH)D increases by 0.28 ng/mL over 5 mo on a given supplemental regimen. Note that a steady state appears to be achieved after ≈ 90 d of each dose tested (2, 3). Thus, doses of 400 IU (10 μ g), 1000 IU (25 μ g), 4000 IU (100 μ g), and 10 000 IU (250 μ g) vitamin D/d for 5 mo will result in theoretical increases in circulating concentrations of 2.8, 7.0, 28, and 70 ng 25(OH)D/mL, respectively, all of which values are in the normal range of circulating concentrations according to reference data (20). In the study by Heaney et al (3), not one case of hypercalcemia or hypercalciuria was observed. The data from the studies of Vieth et al (2) and Heaney et al (3) are summarized in **Table 1**.

We are conducting an ongoing study that involves the supplementation of lactating mothers with 2000 IU (50 μ g; $n = 9$) or 4000 IU (100 μ g; $n = 9$) vitamin D/d for 3 mo. Our preliminary data show increased mean (\pm SD) circulating 25(OH)D concentrations in the 2000-IU/d group (from 27.6 ± 9.8 to 36.1 ± 7.0 ng/mL) and in the 4000-IU/d group (from 32.6 ± 6.9 to 44.5 ± 11.4 ng/mL), all of which are within the normal reference range (38). We note that the breastfeeding infants of these mothers have a substantially improved nutritional vitamin D status because of the transfer of vitamin D into the mother's milk. Circulating 25(OH)D concentrations in the infants of mothers receiving the 4000-IU/d dose increased into the normal range after only 3 mo of breastfeeding (38).

Given the results of more recent scientific studies that evaluated high-dose vitamin D supplementation, it appears that the current RDA, DRI, LOAEL, and NOAEL for adults were based on limited scientific methods and small sample sizes and, therefore, are misleading and potentially harmful. New scientific evidence, including a study by the Centers for Disease Control and Prevention (1), suggests that the DRI for vitamin D should be much higher to achieve adequate nutritional vitamin D status, especially in the African American population because of their darker pigmentation. Further studies are necessary to determine the optimal therapeutic doses of vitamin D during pregnancy and lactation. Given the scientific data that are accumulating about the need for a higher DRI for vitamin D, how does one reconcile past concerns about vitamin D toxicity and hypervitaminosis D?

The first step is to define hypervitaminosis D and to examine the medical literature describing these medical conditions.

HYPERVITAMINOSIS D

Nutritional hypervitaminosis results when pharmacologic doses of vitamin D are consumed for a prolonged period of time and is defined by a large increase in circulating 25(OH)D concentrations (4). The exact amount of vitamin D required to induce toxicity, ie, the amount ingested over a given period of time, is unknown in humans. However, Vieth (4) suggests that this amount is 20 000 IU/d (500 $\mu\text{g}/\text{d}$). In our experience, the amount of circulating 25(OH)D that induces toxicity would have to exceed 100 ng/mL. Eventually, as circulating 25(OH)D increases to toxic concentrations, the classic situation of hypercalciuria, hypercalcemia, and, finally, extraskeletal calcification becomes evident. Hypercalciuria due to excessive vitamin D intakes is always accompanied by circulating 25(OH)D concentrations > 100 ng/mL (39–41). To attain circulating 25(OH)D concentrations that exceed 100 ng/mL, a daily vitamin D intake well in excess of 10 000 IU/d (250 $\mu\text{g}/\text{d}$) for several months would be required (3). Vieth (4) estimates that the physiologic limit for daily vitamin D intake is 250–500 μg (10 000–20 000 IU/d). This amount also makes sense from a physiologic standpoint because this daily vitamin D load (10 000–20 000 IU) would be easily achieved from ultraviolet (UV) light–induced cutaneous synthesis in subjects of all races who work outside in sun-rich environments (22–25). Hypervitaminosis D is a serious, albeit very rare, condition. However, hypervitaminosis D has never occurred when physiologic amounts of vitamin D are ingested. In addition, no case of hypervitaminosis D from sun exposure has ever been reported.

HIGH-DOSE VITAMIN D SUPPLEMENTATION IN INFANTS

The concern regarding excessive vitamin D supplementation during infancy came to the forefront in post-World War II Britain. During that time, it was the practice to supplement each quart (0.95 L) of milk with 1000 IU (25 μg) vitamin D and to fortify many foodstuffs, such as cereals, bread, and flour, with vitamin D (42). It was calculated that most of the infants in Great Britain at that time received between 2000 and 3000 IU (50–75 μg) vitamin D/d (42). In many cases, it could have been much higher because of indiscriminate vitamin D supplementation. Thus, the highest doses that some infants received during this period will never be known because blood concentrations of vitamin D could not be assessed at that time. The indiscriminate use of vitamin D during this time was blamed for a dramatic increase in infantile idiopathic hypercalcemia (43); undoubtedly, uncontrolled vitamin D intakes from a variety of sources contributed to this outbreak. However, 2 important issues remain. First, the actual amount of vitamin D ingested by these infants who were afflicted with idiopathic hypercalcemia will never be known. Second, the contribution of other unknown underlying diseases, such as Williams syndrome, to the idiopathic hypercalcemia will remain unknown.

There is a model of “controlled” high-dose vitamin D supplementation (ie, a supplement given from a single source) during infancy that did not show the problems encountered in Britain. In Finland, from the mid-1950s until 1964, the recommended intake

of vitamin D for infants was 4000–5000 IU/d (100–125 $\mu\text{g}/\text{d}$) (44). In 1964 it was reduced to 2000 IU/d (50 $\mu\text{g}/\text{d}$), and in 1975 it was further reduced to 1000 IU/d (25 $\mu\text{g}/\text{d}$) (44). In 1992, on the basis of the US RDA (5), the dose was reduced again to 400 IU/d (10 $\mu\text{g}/\text{d}$). Under this controlled supplementation regimen, even at the highest intakes, neither idiopathic infantile hypercalcemia nor any other health problem was ever described. However, what was described in a retrospective study was a dramatic decrease in type 1 diabetes later in life in infants who received high-dose daily vitamin D supplementation (44).

HYPERVITAMINOSIS D AS A CAUSE OF SUPRAVALVULAR AORTIC STENOSIS SYNDROME: AN ERRONEOUS ASSOCIATION

Because of the British experience with idiopathic infantile hypercalcemia attributed to hypervitaminosis D, a terribly inaccurate association occurred that had a profound effect on the potential of vitamin D supplementation, not only during infancy but also during pregnancy. In 1963, Black and Bonham-Carter (45) recognized that elfin facies observed in patients with severe idiopathic infantile hypercalcemia resembled the peculiar facies observed in patients with supravalvular aortic stenosis (SAS) syndrome. Shortly thereafter, Garcia et al (46) documented the occurrence of idiopathic hypercalcemia in an infant with SAS who also had peripheral pulmonary stenosis, mental retardation, elfin facies, and an elevated blood concentration of vitamin D. This is an interesting observation because, in 1964, when the article was published, there were no quantitative means of assessing circulating concentrations of vitamin D. In fact, at that time, it was not even proven that vitamin D was further metabolized within the body. By 1966 vitamin D was viewed by the medical community as the cause of SAS syndrome (42, 47). As a result of the theory that maternal vitamin D supplementation during pregnancy caused SAS syndrome (48, 49), animal models were developed to show that toxic excesses of vitamin D during pregnancy would result in SAS (reviewed in the next section) (50–62). In these earlier cases (42, 45–49), vitamin D had nothing to do with the etiology of SAS. What was described as vitamin D–induced SAS syndrome is now known as Williams syndrome (63). Unfortunately, a low vitamin D intake during pregnancy is still associated with SAS.

Williams syndrome is a severe genetic affliction related to elastin gene disruption (64) that is caused by deletion of elastin and contiguous genes on chromosome 7q11.23. This syndrome is characterized by multiorganic involvement (including SAS), dysmorphic facial features, and a distinctive cognitive profile (64). Such patients often exhibit abnormal vitamin D metabolism, which makes them susceptible to bouts of idiopathic hypercalcemia (65–70). This relation was suspected as early as 1976 (71). Subsequently, it was shown that children with Williams syndrome exhibit an exaggerated response of circulating 25(OH)D to orally administered vitamin D (65). Thus, the fear of vitamin D–induced SAS is based on studies that are no longer valid yet continue to be cited.

ANIMAL MODELS OF VITAMIN D TOXICITY DURING PREGNANCY

As mentioned previously, animal models of vitamin D toxicity during pregnancy developed in the 1960s and 1970s were used to

study vitamin D–induced SAS syndrome in humans (50–62). These animal models, almost without exception, focused on feeding or injecting rodents, rabbits, or pigs with toxic concentrations of vitamin D or on administering the active form of vitamin D (1,25-dihydroxyvitamin D) and assessing the biological consequences. The results arising from vitamin D–induced hypercalcemia were devastating: extraskeletal calcifications of the aorta (a defect not observed in Williams syndrome) and other tissues that were usually followed by death. In most of these studies, the animals received 200 000–300 000 IU (5000–7500 μg) vitamin D/kg body wt to elicit these horrendous effects. An equivalent dose to a 60-kg human would be 15 000 000 IU/d (375 000 $\mu\text{g}/\text{d}$). In fact, toxic amounts of vitamin D have been used as a rodenticide, as an alternative to warfarin. However, to achieve the same results in humans, millions of units of vitamin D would have to be ingested.

Two relatively recent publications dealing with vitamin D supplementation during pregnancy and fetal cardiac abnormalities that used animal models need to be addressed. The first of these articles was published in 1985 in a Japanese science journal (59). These investigators fed 2 pregnant pigs different doses of vitamin D. One pig was fed a nearly vitamin D–deficient diet, and the other pig was fed a diet with a relatively normal vitamin D content. The piglets from the mother fed the nearly vitamin D–deficient diet exhibited borderline hypovitaminosis D [15 ng/mL circulating 25(OH)D], whereas piglets from the other sow had normal circulating concentrations of 25(OH)D. The authors attempted to relate normal circulating 25(OH)D concentrations in the piglets to coronary arterial lesions and made the inference that this could be the reason why Americans have a high rate of coronary disease. This study lacked the power to detect any statistically significant differences.

Only one other study has been reported that attempts to repeat the results of the porcine study with the use of a rat model (62). In this study, investigators fed pregnant rats the hormonal form of vitamin D, 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], directly. The intake of 1,25(OH)₂D₃ by these pregnant rats was shown to influence aortic structure, function, and elastin content. These investigators collected blood using EDTA, a calcium-chelating agent, as an anticoagulant; as a consequence, circulating calcium could not even be measured! Thus, clinically significant hypervitaminosis D was not evident on the basis of its primary effect—hypercalcemia, which invalidated the study. In addition, because the authors used the hormonal form of vitamin D [1,25(OH)₂D₃], the study was considered to be pharmacologic and, thus, did not represent or recapitulate normal physiology in humans, ie, the hormonal form bypassed the body's normal regulation of the conversion of 25(OH)D to 1,25-dihydroxyvitamin D. Thus, these animal studies have no bearing on normal human nutrition.

ANIMAL MODELS OF VITAMIN D DEFICIENCY DURING PREGNANCY

Conversely, other studies have shown just how important adequate nutritional intakes of vitamin D are to skeletal, cardiovascular, and neurologic development in experimental animals (72–76). Weishaar and Simpson (72) showed that lengthy periods of vitamin D deficiency in rats are associated with profound changes in cardiovascular function, including increases in cardiac and vascular muscle contractile function. These investiga-

tors later showed by histologic examination that ventricular muscles from vitamin D–deficient rats showed a significant increase in extracellular space (73). Morris et al (74) reported that low maternal consumption of vitamin D retarded metabolic and contractile development in the neonatal rat heart. The authors concluded that low maternal vitamin D intakes result in a general, but significant, slowing of neonatal cardiac development.

It has been suggested that vitamin D could be involved in brain function and neurodevelopment (77, 78). A recent study provides startling evidence with respect to the consequences of vitamin D deficiency on the neurodevelopment of the fetus during pregnancy in a rat model (75). Pups born to vitamin D–deficient mothers had cortex abnormalities, enlarged lateral ventricles, and more cell proliferation throughout the brain. Furthermore, the rats showed a reduction in the brain content of nerve growth factor and glial cell line–derived neurotrophic factor and a reduction in the expression of p75^{NTR}, the low-affinity neurotrophin receptor. These findings suggest that low maternal vitamin D has important ramifications for the developing brain.

It has been known for decades that adequate vitamin D is required for normal skeletal development. The importance of vitamin D to skeletal integrity has been shown in a study involving rodents (76). This study showed that hypovitaminosis D during pregnancy impaired endosteal bone formation, which resulted in trabecular bone loss, and concluded that vitamin D is indispensable for normal bone mineralization during the reproductive period in rats.

HUMAN STUDIES INVOLVING PHARMACOLOGIC DOSES OF VITAMIN D DURING PREGNANCY

A study in human subjects involved the administration of 100 000 IU vitamin D/d (2.5 mg/d) throughout pregnancy to hypoparathyroid women to maintain serum calcium (79, 80). The infants from the larger of the 2 studies (79; $n = 15$) underwent an examination of facial structure, a palpation of pulses, and an auscultation for significant murmurs or bruits over the entire chest, back, abdomen, and peripheral vessels. Furthermore, the children were examined at ages ranging from 6 wk to 16 y. Many of the children were examined several times over a 4-y period. None of the children had any of the craniofacial stigmata associated with infantile hypercalcemia. Specifically, none had micrognathia or evidence of SAS, pulmonary stenosis, or other detectable cardiovascular anomalies. Greer et al (80) showed that an infant delivered from a hypoparathyroid mother who had received 100 000 IU (2.5 mg) vitamin D/d had circulating 25(OH)D concentrations of 250 ng/mL at birth; yet, this infant was perfectly normal and healthy. This woman again became pregnant and subsequently delivered another healthy term infant. Thus, there is no evidence in humans that even a 100 000 IU/d dose of vitamin D for extended periods during pregnancy results in any harmful effects. Pharmacologic doses of 1,25(OH)₂D₃ given to a woman to treat hypocalcemic rickets during her pregnancy produced no ill effects on the developing fetus; these infants were specifically evaluated for elfin facies and SAS (81).

VITAMIN D SUPPLEMENTATION DURING HUMAN PREGNANCY

The Cochrane Library recently issued a review of vitamin D supplementation during pregnancy (82) and identified 7 studies

on the topic in question (32–34, 36, 83–85); however, only 4 reported clinical outcomes (32, 33, 83, 84). The Cochrane review concluded that there is not enough evidence to evaluate the requirements and effects of vitamin D supplementation during pregnancy. Presented below are the clinically relevant studies offered by the Cochrane review plus 3 additional studies identified by our group.

Initial vitamin D supplementation studies during pregnancy were carried out in the early 1980s. Brooke et al (32), who studied British mothers of Asian descent, found a greater incidence of small-for-gestational-age infants born to mothers who received placebo than in mothers who received 1000 IU (25 μg) vitamin D₂/d during the final trimester of pregnancy. Neonates in the placebo group also had a greater fontanelle area than did the supplemented group. It must be noted that the placebo group in this study showed profound hypovitaminosis D. Follow-up studies by Brooke et al (83) were conducted in Asian mothers who again were provided with either placebo or 1000 IU vitamin D₂/d during the last trimester of pregnancy. The follow-up data provided evidence that, during the first year of life, the infants of the maternal placebo group gained less weight and had a lower rate of linear growth than did the infants of the maternal supplemented group.

Cockburn et al (33) undertook a large vitamin D supplementation study of > 1000 pregnant subjects in the United Kingdom who were supplemented with 400 IU (10 μg) vitamin D₂/d or received a placebo from week 12 of gestation onward. At this level of supplementation, serum concentrations of 25(OH)D in the supplemented group were only slightly higher than those in the placebo group. A defect in dental enamel formation was observed in a higher proportion of the children at 3 y of age in the maternal placebo group. Maxwell et al (84) conducted a double-blind trial of vitamin D (1000 IU/d) during the last trimester of pregnancy in Asian women living in London. They found that the supplemented mothers had greater weight gains and, at term, had significantly higher plasma concentrations of retinol-binding protein and thyroid-binding prealbumin, which indicated better protein-calorie nutrition. Almost twice as many infants of the unsupplemented group weighed < 2500 g at birth and had significantly lower retinol-binding protein concentrations than did infants of the supplemented mothers. Brunvard et al (86) followed 30 pregnant Pakistani women who were free of chronic diseases and had uncomplicated pregnancies. Nearly all of the women had low (< 15 ng/mL) circulating 25(OH)D concentrations, and nearly 50% exhibited secondary hyperparathyroidism. The maternal circulating parathyroid hormone concentration was inversely related to the neonatal crown-heel length. These authors concluded that maternal vitamin D deficiency affected fetal growth through an effect on maternal calcium homeostasis.

HOW DOES VITAMIN D SUPPLEMENTATION DURING PREGNANCY AFFECT THE NUTRITIONAL VITAMIN D STATUS IN BOTH MOTHER AND FETUS?

This is an important question that remains to be addressed. In the United States, the current DRI for vitamin D during pregnancy is 200–400 IU/d (5–10 $\mu\text{g}/\text{d}$). However, supplementation of mothers, by Cockburn et al (33), with 400 IU vitamin D/d during the last trimester of pregnancy did not significantly increase circulating 25(OH)D concentrations in the mothers or their infants at term. This finding agrees with current data in

healthy men published by Heaney et al (3). Supplementation with 1000 IU (25 μg) vitamin D/d during the last trimester of pregnancy has produced mixed results. The initial study by Brooke et al (32) described a dramatic increase, 50–60 ng/mL, in circulating 25(OH)D in both mothers and neonates at term (Table 1). However, these results are highly suspect in light of later and current work (2, 3, 35, 37, 38) and are consistent with a dose response obtained after consumption of 10 000 IU (250 μg) vitamin D/d for 3 mo. There also is a possibility that the 25(OH)D assay method used in this study was flawed, as was common during this early period of investigation.

Mallet et al (35) reported that vitamin D supplementation (1000 IU/d, or 25 $\mu\text{g}/\text{d}$) during the last trimester of pregnancy resulted in an increase in circulating 25(OH)D concentrations of only a 5–6 ng/mL in maternal and cord serum. In the most recent study, by Datta et al (37), 160 pregnant minority women in the United Kingdom were provided with 800–1600 IU (20–40 μg) vitamin D/d for the duration of their pregnancy. Using modern assay technology for the measurement of circulating 25(OH)D concentrations (21), these investigators found a mean (\pm SD) increase in circulating 25(OH)D concentrations (ng/mL) of from 5.8 ± 0.9 at the beginning of pregnancy to 11.2 ± 6.3 at term after vitamin D supplementation. A normal serum circulating 25(OH)D concentration in the United States is considered to be > 15 ng/mL (20). However, a circulating concentration of 15 ng 25(OH)D/mL is marginal for nutritional vitamin D status (10). In other words, mothers who were vitamin D deficient at the beginning of their pregnancy were still deficient at the end of their pregnancy after being supplemented with 800–1600 IU vitamin D/d throughout their pregnancy. In other words, mothers who were vitamin D deficient at the beginning of their pregnancy were still deficient at the end of their pregnancy after being supplemented with 800–1600 IU vitamin D/d throughout their pregnancy. This result is precisely what the regression analysis from Heaney et al (3) predicted would happen at this vitamin D intake, and this is a problem (Table 1). The results of this study again point out that the DRI for vitamin D during pregnancy is grossly inadequate, especially in ethnic minorities. The data of Vieth et al (2) and Heaney et al (3) and our own data in lactating women (38) suggest that doses exceeding 1000 IU vitamin D/d (2000–10 000 IU/d) are required to achieve a robust normal concentration of circulating 25(OH)D. This should be the goal of future research in this area.

MATERNAL AND CORRESPONDING FETAL VITAMIN D CONCENTRATIONS

Many studies in human subjects have shown a strong relation between maternal and fetal (cord blood) circulating 25(OH)D concentrations (87–90). Our group showed that vitamin D status at birth is closely related to that of the mother and is greatly influenced by race (90). The data showed that the fetus at birth (cord blood) will contain \approx 50–60% of the maternal circulating concentrations of 25(OH)D. This relation appears to be linear, even at pharmacologic intakes of vitamin D (80). With respect to the more polar metabolites of vitamin D, a similar (but lesser) relation is observed between mother and fetus (90). Interestingly, there appears to be little, if any, relation with respect to the parent vitamin, vitamin D (90). This lack of placental transfer of parent vitamin D from mother to fetus was also observed in a porcine experimental animal model (91). Thus, in the human fetus, vita-

min D metabolism in all likelihood begins with 25(OH)D. As a result, the nutritional vitamin D status of the human fetus and neonate is totally dependent on the vitamin D stores of the mother (90); thus, if the mother has hypovitaminosis D, her fetus will experience depleted vitamin D exposure throughout the developmental period.

Finally, let us discuss a scenario that occurs thousands of times daily in the United States. A pregnant woman visits her obstetrician, who prescribes prenatal vitamins containing 400 IU (10 μg) vitamin D. The patient and physician both assume that this supplement will fulfill all the nutritional requirements for the duration of the pregnancy. However, in the case of vitamin D, it will not even come close unless the pregnant woman has adequate sun exposure. The woman, especially if African American, and her developing fetus are at high risk of remaining vitamin D deficient during the entire pregnancy (1). Even if the physician were to prescribe a vitamin D supplement of 1000 IU/d (25 $\mu\text{g}/\text{d}$), the mother would likely remain vitamin D deficient (35, 37). As scientists and health care providers, we simply cannot accept this any longer. The true requirement for vitamin D during pregnancy must be determined scientifically.

VITAMIN D SUPPLEMENTATION DURING LACTATION

Scientific data pertaining to vitamin D supplementation during lactation in the humans is even scarcer than data on vitamin D supplementation during pregnancy. As during pregnancy, an arbitrary DRI has been set at 400 IU/d (10 $\mu\text{g}/\text{d}$). For the reasons stated in the previous sections, we consider a 400-IU/d vitamin D supplement to lactating mothers to be inadequate. This level of supplementation will do nothing to increase or even sustain the nutritional vitamin D status of mothers or their breastfeeding infants. We believe that vitamin D supplementation of lactating mothers has a dual purpose: 1) to increase the nutritional vitamin D status of the mother and 2) to improve the vitamin D nutriture of the breastfeeding infant. A maternal intake of 400 IU vitamin D/d will accomplish neither of these goals. To our knowledge, not a single prospective study has been performed to evaluate the effects of supplementing lactating mothers with 400 IU vitamin D/d. In other words, we have no idea what effect this dose would have on the nutritional vitamin D status of the mother or her infant. However, on the basis of the regression model of Heaney et al (3), supplementation of pregnant women with 400 IU vitamin D/d would only increase circulating 25(OH)D concentrations by 2.8 ng/mL after 5 mo.

We found only a single study that prospectively examined vitamin D supplementation during lactation (92). In this study, lactating mothers were supplemented with either 1000 IU (25 μg) or 2000 IU (50 μg) vitamin D/d for 15 wk. Increases in circulating 25(OH)D concentrations during this period of supplementation were 16 and 23 ng/mL in the 1000- and 2000-IU groups, respectively. We conducted preliminary studies in which lactating mothers were supplemented with 2000 and 4000 IU vitamin D/d for 3 mo (38). Our data also showed an increase in circulating maternal 25(OH)D concentrations, although not as pronounced as those observed by Ala-Houhala et al (92). It is clear that larger, more detailed studies are required to determine the vitamin D requirements of lactating mothers.

VITAMIN D CONTENT OF HUMAN MILK AND FACTORS AFFECTING THIS CONTENT


In the past, human milk was thought to be an adequate source of antirachitic activity for neonates and growing infants. Even before the discovery of vitamin D, McCollum et al (93) and Park (94) stated that rickets was due to the deprivation of sunlight and dietary factor X. They observed that factor X was found in "good breast milk" and cod liver oil and that, although rickets did develop in breastfed children, it was rarely as severe as in artificially fed infants. These investigators did not know that the source of vitamin D in the mother's milk was the mother's exposure to the sun, which cutaneously generated large amounts of vitamin D. Ultimately, this solar-derived vitamin D ended up in the mother's milk for the infant. Early attempts to quantify the antirachitic potential of human milk were crude and yielded little information (95–97). For a time, it was believed that vitamin D sulfate was responsible for the antirachitic activity in human milk (98, 99); however, this was later shown not to be the case (100).

In the 1980s, the antirachitic activity of human milk was defined with sensitive assay technology to be 20–70 IU/L (101–103). Furthermore, almost all of the activity was attributable to vitamin D and 25(OH)D. These studies also showed that dietary maternal vitamin D supplementation and ultraviolet (UV) light exposure increase the vitamin D content of human milk (102, 104, 105). Specker et al (106) determined that the antirachitic activity of human milk was lower in African American than in white mothers. This difference was attributed to variations in the dietary intake of vitamin D and exposure to UV light. As presented earlier, the woman with hypoparathyroidism treated with 100 000 IU (2.5 mg) vitamin D/d for the maintenance of her plasma calcium concentration throughout pregnancy delivered a healthy child at term and then breastfed her infant (80). Analysis of breast milk from this mother showed it to contain ≈ 7000 IU/L of antirachitic activity. From our current studies involving lactating mothers receiving up to 4000 IU (100 μg) vitamin D/d, we showed elevations in the antirachitic activity of some of the mothers' milk to > 400 IU/L (38). Thus, it is clear that the vitamin D content of human milk can be influenced by maternal diet, UV light exposure, or both. If a lactating mother has a limited exposure to UV light, a limited vitamin D intake [such as occurs at the current DRI of 400 IU/d (10 $\mu\text{g}/\text{d}$)], or both, the vitamin D content of her milk will be low, especially if she has darker skin pigmentation.

EFFECT OF BREASTFEEDING ON INFANT VITAMIN D STATUS AND ITS RELATION TO NUTRITIONAL RICKETS

Thirty-five years ago the incidence of nutritional rickets was thought to be disappearing (107). Many reports since then, however, indicate that this is not the case (108–112). Most of the cases of rickets reported in the last few years have been in darkly pigmented infants who had been breastfed exclusively. Hypovitaminosis D in breastfed infants is also a severe problem in sun-rich environments, such as the Middle East (113). This hypovitaminosis D results because sun exposure to both mothers and infants is extremely limited. Furthermore, dietary supplementation in this population is not a common practice.

From the prior discussion in this report, it is clear that the antirachitic activity of human milk is variable and is affected by

season, maternal vitamin D intake, and race. How then is the nutritional vitamin D status of neonates and infants affected if they are exclusively breastfed? Cancela et al (114) reported that circulating 25(OH)D concentrations in breastfed infants are directly related to the vitamin D content of the mothers' milk. Available evidence indicates that if the vitamin D status of the lactating mother is adequate, her breastfeeding infant will maintain a "minimally normal" nutritional vitamin D status. The best example of this was presented by Greer and Marshall (115). These investigators found that white infants who were exclusively breastfed during the winter in a northern climate maintained a "minimally normal" vitamin D status for 6 mo. Note, however, that the circulating 25(OH)D concentrations in the breastfeeding infants from this study actually decreased as the study progressed. This decrease occurred despite a maternal vitamin D intake of ≈ 700 IU/d ($17.5 \mu\text{g/d}$) (115). In contrast, a Finnish study showed that maternal supplementation with 1000 IU ($25 \mu\text{g}$) vitamin D/d resulted in a minimal increase in circulating 25(OH)D concentrations in breastfeeding infants (36). These same investigators repeated a similar study with 2000 IU ($50 \mu\text{g}$) vitamin D/d and found that the vitamin D status of the breastfeeding infants improved significantly (92). Our group recently performed similar studies, supplementing lactating women with 2000 or 4000 IU vitamin D/d for 3 mo (38). We found that high-dose maternal vitamin D supplementation not only improves the nutritional vitamin D status of breastfeeding infants but also elevates the maternal concentrations into the mid-normal range. Thus, a dual benefit is achieved from high-dose maternal supplementation. It is noteworthy that in the Finnish study, the authors added a disclaimer, "A sufficient supply of vitamin D to the breastfed infant is achieved only by increasing the maternal supplementation up to 2000 IU/d. Such a dose is far higher than the RDA [DRI] for lactating mothers [and therefore] its safety over prolonged periods is not known and should be examined by further study." This point of concern was valid when this study was conducted in 1986 (92); however, on the basis of the current findings of Vieth et al (2) and of Heaney et al (3)—which showed that vitamin D intakes $\leq 10\,000$ IU/d ($250 \mu\text{g}$) are safe for prolonged periods (up to 5 mo)—we believe that it is time to reexamine the understated DRI of vitamin D for lactating mothers. This work is now being conducted in our clinics and laboratory. 

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